ULTRA-MICROSCOPIC VISION.

IN NATURE, November 5, 1908, a short paragraph appeared in reference to a letter received from Mr. G. V. Raman, of the Science Association Laboratory, Calcutta, referring to a method of dark ground illumination for the microscope. From it, and from a subsequent communication on the same subject, it would appear that the subject of dark ground illumination and ultra-microscopic illumination may in certain directions give rise to controversy, and result in some confusion of thought.

It is unquestioned that any method of microscopic illumination in which the direct axial beam of light is cut out, and where, therefore, a grazing or oblique illumination is obtained, may result in making visible some particles that are beyond the limits of a microscope illuminated by ordinary methods.

It must at once be admitted that it is difficult to define the exact boundary beyond which objects may be said to be ultra-microscopic. To appreciate this point, it is necessary to refer very briefly—owing to the limits imposed in such a short article as this—to the wide difference between the limits of microscopic resolution and microscopic visibility.

To define the limits of resolution of the microscope is not difficult, as this is purely a function of the numerical aperture of the objective. The limits in this direction have been accurately determined, and practically agree in theory and practice. In the case of periodic structure, such as in diatoms, or in mechanically-ruled plates such as Grayson's rulings, this resolving limit can be found by multiplying the numerical aperture of the objective by 80,000 when monochromatic green light is used, and illumination is by a solid axial cone of light. This means approximately that lines of more than 120,000 to the inch would be beyond the limit of resolution when using an objective with N.A. 1·40, the largest aperture generally available at present; or that two points lying closer together than the distance between these would be evident, not as two separate images, but would so overlap as to appear as one.

This, however, is by no means the limit of visibility, and Lord Rayleigh states that isolated objects, or two bright areas separated by a dark line, may be seen if the dark line is as narrow as 1/16, and under certain conditions 1/32, of a wave-length of light, although the resulting image does not of necessity represent the actual appearance of the object. The flagellum of a bacterium, for instance, may be much beyond the limit of resolution, but is visible because it is an isolated object.

Another factor is the intensity of the incident light, and there is some reason to conclude that any relatively isolated object may be visible if it is illuminated with sufficient intensity, and can reflect light enough for the eye to appreciate. A keen observer will see in a microscopic image all structure that the best objectives can reproduce with a magnification of little more than 750 diameters, although it may be convenient to amplify the image beyond this to facilitate observation. Objects that are smaller than this limit of resolution are generally referred to as ultra-microscopic, although it is obvious that the term is not always justified. It is clear, therefore, that to define the meaning of the term "ultra-microscopic" is by no means simple, and especially in view of the fact that most methods of dark ground illumination do result in the formation of images that are not seen in other ways.

Illumination in the microscope by means of light projected at various angles to the optical axis has been common for very many years. The writer has used, for example, an oil immersion paraboloid made by Messrs. Swift and Son, probably very soon after the introduction of oil-immersion objectives, say about 1875, and the results to be obtained with it compare favourably with those of more recent introductions. At various times, other methods have been introduced. The simplest, and one of the earliest, was what is known as a "spot lens," also a dry paraboloid, and the arrangement by which the ordinary substage condenser may be utilised. In each of these a blackened stop of suitable size is placed beneath the optical portion of the illuminating system in such a position that the central axial rays are obstructed, and no light directly enters the objective. Only light refracted or reflected by the object reaches the objective, and the former, therefore, shows up brightly illuminated on a more or less dark background. With each of these arrangements only objectives of relatively low aperture can be used.

Other methods are those in which a stop is placed above the posterior combination of the objective, or the very ingenious arrangement suggested by Mr. J. W. Gordon, in which the stop, in this case a small globule of mercury, is placed above the eye-piece in the position occupied by the Ramsden disc. The closest approximation to the modern ultra-microscope of Siedentopf is the type of dark ground illumination in which the light is reflected so that it impinges upon the object at right angles to the optical axis of the microscope, but in none of these is any attempt made to confine the illuminating beam to the area under observation.

In 1903 an entirely new method of rendering visible ultra-microscopic particles was brought out by Siedentopf, and arose out of some investigations being made on various shades of ruby glass. As is probably well known, the colouring of ruby glass is dependent on small particles of gold, the dimensions of which approach in size to that of a molecule. If examined under an ordinary microscope and by ordinary methods of illumination, or by any method of dark ground illumination, even with the very best objectives there is no indication of the presence of any isolated particles. But, by a method of projecting a very thin cone of light at right angles to the optical axis of the microscope, and exactly on the spot under observation only, they were able clearly to observe diffraction discs which became visible, and arose from each individual particle of gold in the ruby glass.[1] The method is therefore one entirely depending on the arrangement and exact control of the illumination. The initial intensity of the illuminant must be high, so that only the electric arc or sunlight is suitable.

In general, the illumination of the object is accomplished by projecting the image of a very narrow precision slit, which is constructed in a similar manner to those used on fine spectroscopes so that both the length and breadth of the slit can be varied and exactly determined on to the object. The apparatus is arranged so that a very powerful beam of light is projected through the slit and focussed by a suitable optical arrangement so that the apex of the cone of rays falls exactly on the spot in the object where the objective is focussed, and so that no particles lying above or below this spot are illuminated. Consequently, only the particles in the field of view are sufficiently luminous to form an image in the microscope, and no particles lying outside this field can diffuse light and interfere with the formation of the image.

This latter disadvantage is common to all other methods of dark ground illumination, and it is in this respect chiefly, if not entirely, that the Siedentopf

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[1] See Journal of the Royal Microscopical Society, 1903, p. 573.

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method differs from all others. It would happen with a powerful source of light, unless the apparatus were thus arranged, that so many small particles would be illuminated that the diffraction discs would overlap and simply produce a general diffusion of light, and but very few individual particles, and of these only the more isolated ones, would therefore be visible. This is exactly what happens with any ordinary method of dark ground illumination where the particles are numerous or where they are distributed throughout a considerable area. In the Siedentopf method, where the light is simply concentrated on one spot in the field alone, little or no interference between the diffraction discs results, internal reflections between the components of the optical system are reduced to a minimum, and consequently an image is obtained which under other conditions would be impossible.

Considerable developments have taken place recently in methods of dark ground illumination. It is now possible to view such objects as bacteria with ease, without resorting to the excessive staining that is unfortunately so prevalent, enabling them also to be observed while in the living condition. The most satisfactory of these is the parabolic illuminator recently introduced by Messrs. Zeiss (Fig. 1). It may easily be said, and it is perfectly true, that the parabolic illuminator, either dry or oil immersion, is no novelty, but the one made by Messrs. Zeiss has been developed along scientific lines, and is the result of careful computation, whereas those made in the earlier days of microscopy were largely the result of chance, or at least trial and error, and it was a fortunate circumstance if they gave a result which was entirely satisfactory.

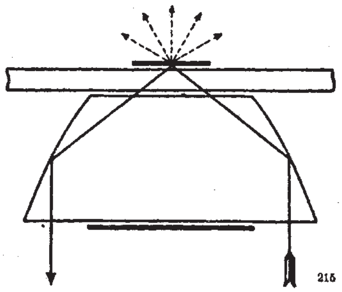

FIG. 1.

In the case of the Zeiss parabolic illuminator, the light is so reflected from the internal surface of the paraboloid that the annular cone of rays is projected, and has its focal point exactly where the objective is focussed. It would not, of course, give the same results as Siedentopf's method with such objects as ruby glass or colloidal solutions, but for observing minute living bacteria or similar transparent objects it leaves little to be desired. The illuminating rays, too, are exactly confined within the limits of a numerical aperture of 1·1 to 1·4, so that, if using, say, a 4-millimetre apochromatic objective with an aperture of 0·95, no rays would enter directly, and it would only be those reflected or refracted by the object that would pass into the objective at all, the objects, in fact, behaving as self-luminous bodies. This is a definite improvement on the method of introducing a stop into the substage condenser, so that the central rays are blocked out, and only the peripheral rays are allowed to pass, as there is much less spherical and chromatic aberration, the image being to a large extent dependent on reflected light. This appliance will render particles visible that might be termed ultra-microscopic, and in any solution or preparation of bacteria in water a great number of diffraction discs will be visible that by ordinary direct light could not be seen.

Another method that fulfils its purpose is the reflecting condenser made by Messrs. Leitz, of Wetzlar. In this, two reflecting surfaces, the one internal and the other external, as shown in Fig. 2, are so shaped as almost completely to unite the rays at a point P. The light enters from below, and takes the direction as shown by the dotted lines, ultimately converging on the point P, which is the position of the object, and is the focal point of the objective. It is obvious, therefore, that there is no chromatic or spherical aberration. The adjustments for centring are exactly the same as for an ordinary substage condenser, and the optical portion is contained in the mount that slides into the ordinary substage carrier. This apparatus, as well as the Zeiss, requires that the object-slide and the cover-glass shall be of a certain thickness, and cedarwood oil is used between the top surface of the condenser and the slide. It may be used as shown with any dry lens, but the best results are obtained with an apochromat, especially with the 4-millimetre 0·95 N.A. The cone of the illuminating rays is confined within the same limits as the Zeiss apparatus.

It is necessary to remember, however, that because a particle that is invisible by axial illumination becomes visible by oblique light, it does not, therefore, follow that it is ultra-microscopic. Its transparency may be too great, or its refractive index may too nearly coincide with the medium in which it lies for it to be visible by direct light. By oblique illumination a much smaller difference in refractive index between the object and its medium will be sufficient to form an image.

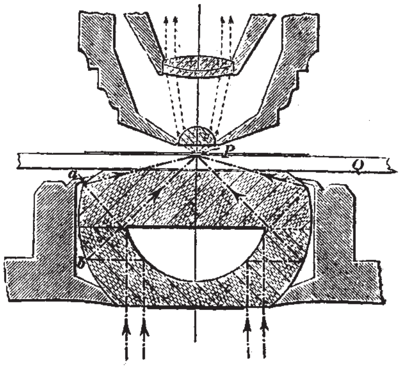

FIG. 2.

It is very difficult within the limits of a short article such as this to go into the matter sufficiently thoroughly to deal with all the points at issue. It may easily be urged that particles that are ultra-microscopic can be made visible by methods other than those described. It is well known that even passing a very powerful beam of light through a darkened room will render visible a large number of particles that cannot be seen by ordinary methods of illumination, and it is more than probable that many of the particles so observed are, in fact, ultra-microscopic. Faraday was able, by projecting a powerful beam of sunlight through a piece of ruby glass, to view the diffraction discs arising from the gold particles in the glass without any microscope at all. The whole question resolves itself really into the necessity of having primarily a sufficiently strong source of light, and so to arrange the incidence of the light that only those particles in the field of view are illuminated. In many arrangements that have been described for microscopic illumination, these two conditions have not been combined, and it is only recently that it has been thoroughly realised that either one without the other will not give the looked-for result.

J. E. BARNARD.

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